Expression of Anionic Sites at the Dermoepibolic Junction

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The emergence of anionic sites during basement membrane zone formation was studied using migrating epidermis in organ culture as a model system.

Ultrastructural investigations using a strongly cationized probe revealed that the heparitinase-sensitive, anionic sites were formed synchronously with the newly built basal lamina after 7 days in culture. *J Invest Dermatol 88:94–96, 1987*

Considerable advances have been achieved in the analysis of the localization of collagenous and noncollagenous components on skin basement membrane zone (sBMZ) (reviewed in [1–3]). Heparan sulfate proteoglycan (HSPG) is a pericellular macromolecule consisting of a core protein bearing heparan sulfate glycosaminoglycan chains, and was found in many tissues as a component of the basement membrane as well as of the cell surface (reviewed in [4]). Antibody prepared against HSPG isolated from the Engelbreth-Holm-Swarm sarcoma matrix was found by immunofluorescence to react with sBMZ [5]. This antigenic distribution was considered to correspond with ultrastructurally demonstrated anionic sites along the epidermal and dermal edges of the basal lamina (BL) [6]. In this location, HSPG played a major role in the regulation of permeability by creation of a charge-selective barrier (reviewed in [7]).

Recently, it has been reported that Schwann cells synthesize 2 major HSPG that differ in size and apparent distribution. Furthermore, the larger (BL-associated) proteoglycan accumulates only when Schwann cells are actively synthesizing BL and the accumulation of the smaller (membrane-associated) proteoglycan is independent of BL production [8]. Nevertheless, detailed information relating to the synthesis of HSPG during sBMZ formation has been rather scarce to date. In the present study, the expression of anionic sites, particularly in relation to that of the BL, was investigated using migrating epidermis in organ culture (epiboly).

MATERIALS AND METHODS

Organ Culture System Organ culture of adult human skin was carried out according to the method of Hintner et al [9]. The specimens were kept at 37°C in a humid atmosphere containing 5% CO_2 in air for 7 days.

Ultrastructural and Tracer Studies The specimens were removed from the culture after 7 days and were stained according to the method reported previously using strongly cationized polyethyleneimine (PEI) as a tracer [6]. For enzyme digestion study, the specimens were digested by 50 units/ml heparitinase in 0.1 M sodium acetate buffer (pH 7.0) at 43°C for 2 h and were stained by the same method. After routine processing, ultrathin sections were observed in a JEM 1200EX electron microscope.

RESULTS

The BL formation along the dermoepibolic junction lagged far behind the advancing tip of the migrating epidermis.

The anionic sites could be visualized in both the dermal and epidermal edges of the continuous area (Fig 1*a*) and end point (Fig 1*b*) of the regenerated BL as small particles (approximately 20 nm in diameter) occurring at regular distances from each other with a center-to-center spacing of approximately 60 nm. They were removed completely by digestion with heparitinase (Fig 1*c*). These results were identical to that of normal sBMZ reported previously [6]. Additionally, they were present on the focal area of regenerated BL subjacent to the hemidesmosome (Fig 1*d*). Occasionally, they could also be detected on the cell surface of basal cells that rested on dermal ground substance and collagen without an intervening BL structure (Fig 1*e*). However, the distances between particles were rather irregular and the size and electron density were reduced compared with the continuous area of regenerated BL, although it was unclear why there were such differences.

DISCUSSION

The distinct components of sBMZ have been noted to re-form utilizing organ culture as a model system. Hintner et al reported that bullous pemphigoid antigens emerged synchronously with the advancing tip of the migrating human epidermal cells up to 7 days of culture, whereas type IV collagen and laminin appeared with considerable delay linking to each other [9]. Stenn et al also reported that in a 48-h culture of mouse skin, migrating epidermal cells contained type V collagen but not type IV collagen [10].

In this study, we have sought to electron microscopically examine the formation of anionic sites in 7-day cultures of normal human skin, in order to better understand their relationships to the BL formation. Ultrastructural studies using a strongly cationized tracer (PEI) demonstrated that the small PEI-positive

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Abbreviations:
BL: basal lamina
HSPG: heparan sulfate proteoglycan(s)
PEI: polyethyleneimine
sBMZ: skin basement membrane zone

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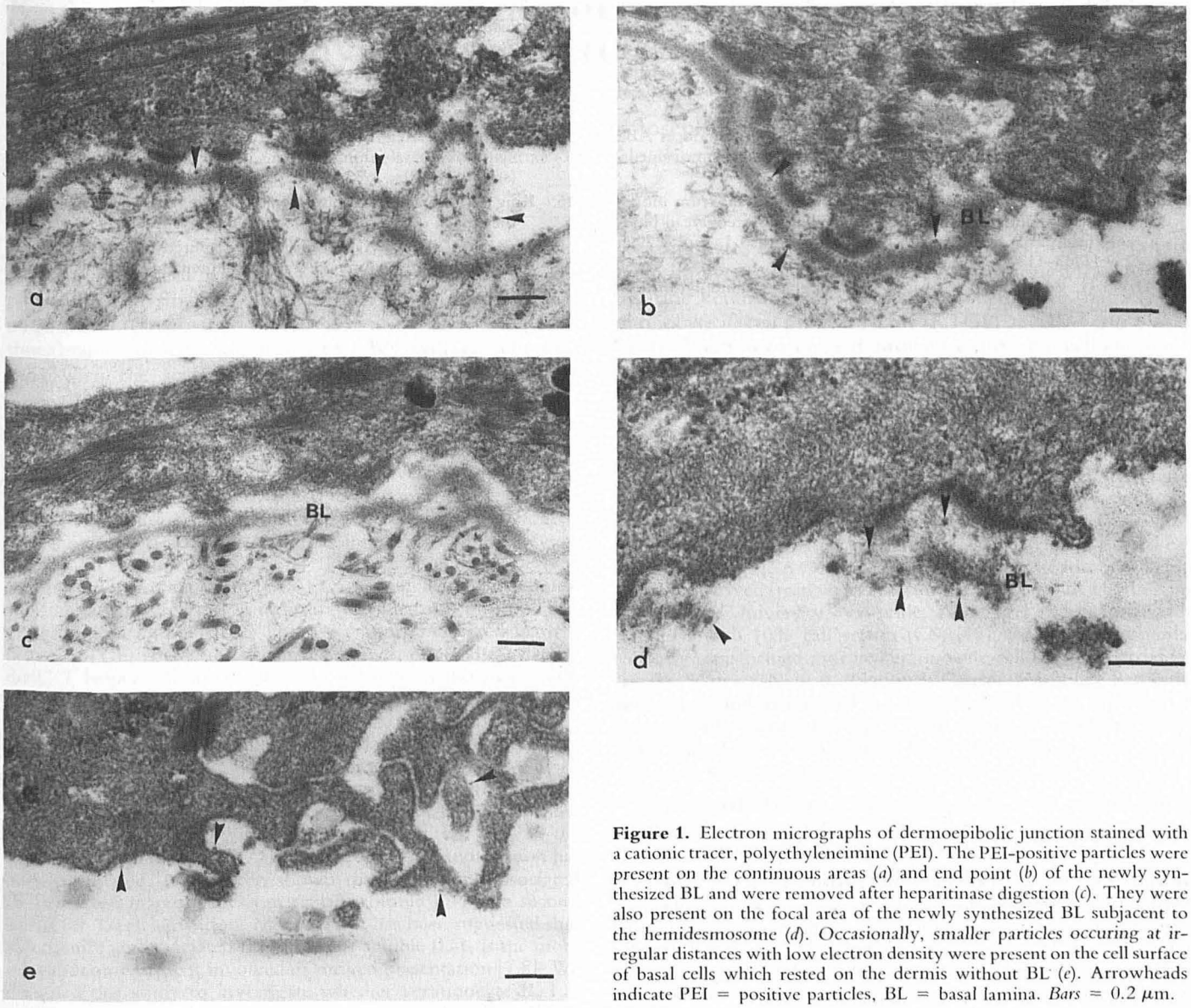

Figure 1. Electron micrographs of dermoepibolic junction stained with a cationic tracer, polyethyleneimine (PEI). The PEI-positive particles were present on the continuous areas (*a*) and end point (*b*) of the newly synthesized BL and were removed after heparitinase digestion (*c*). They were also present on the focal area of the newly synthesized BL subjacent to the hemidesmosome (*d*). Occasionally, smaller particles occuring at irregular distances with low electron density were present on the cell surface of basal cells which rested on the dermis without BL (*e*). Arrowheads indicate PEI = positive particles, BL = basal lamina. *Bars* = 0.2 μm.

(anionic), heparitinase-sensitive particles were present on the continuous areas, end point and focal area of regenerated BL, and basal surface which rested directly on the dermis without BL. Since it has been reported that mouse mammary epithelial cells deposit at their basal surface an extracellular HSPG that binds to type I collagen [11] and that type I collagen fibrils cause these cells to accumulate a BL-like layer [12,13], our findings might suggest that the anionic sites were formed synchronously with BL formation, and that the interaction between the anionic sites and dermal component(s) would be important during sBMZ remodeling.

There was abundant evidence that keratinocytes synthesized several sBMZ components (reviewed in [14]) including sulfated glycosaminoglycans [15,16]. In our present study, however, it remained unclear whether keratinocytes synthesized BL-associated HSPG or what factor(s) were essential for HSPG biosynthesis. It would be interesting in future research to determine which cell type is producing the anionic sites, and what other basement membrane components interact for sBMZ formation.

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